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Ectopic activation of members of the wnt family, such as wnt-1, causes the formation of mammary tumors in mice, providing a model for breast cancer. However, Wnt receptors, essential components mediating Wnt oncogenic functions,had not been identified previously. In *Drosophila*, a member of the Frizzled (Fz) family of seven transmembrane receptors, the DFrizzled 2 (DFz2), was shown to function as a receptor for the Wingless, the ortholog of Wnt-1. This implies that the large family of Fz proteins are likely receptors for Wnt molecules. However, the scarcity of soluble Wnt proteins complicates the study of ligand-receptor relationships and their specificity. We developed an approach in *Xenopus* embryos to persue such study.

In *Xenopus* embryos, the Wingless/Wnt-1 subclass of Wnt molecules induces axis duplication whereas the Wnt-5A subclass does not. This difference could be explained by distinct signal transduction pathways or by a lack of Wnt-5A receptor(s) during axis formation. We found that Wnt-5A induces axis duplication in the presence of hFz5, a member of the Frizzled family of seven transmembrane receptors. Wnt-5A/hFz5 signaling is antagonized by glycogen synthase kinase-3 and by the N-terminal ectodomain of hFz5. These results identify hFz5 as a receptor for Wnt-5A. In addition, we found that a secreted Frizzled related protein, FRP, is an antogonist for Wnt. This study illustrated a general approach for studying Wnt-Fz interactions.

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INTRODUCTION

Wnt genes encode a large family of secreted signaling molecules essential for development and tumorigenesis (1, 2). Of particular interests to human breast cancer research, ectopic activation of certain wnt genes, such as wnt-1, causes mammary carcinogenesis in mice (3). How Wnt-1 induces mammary tumors is not understood. However, given that Wnt molecules are secreted growth factors, it is likely that a receptor-mediated signaling transduction pathway is involved. The aim of this project was to identify Wnt receptors.

In Drosophila, wingless (wg) gene, the ortholog of murine wnt-1, is required for many stages of embryogenesis (4, 5). Interestingly, upon gene transfer wg CDNA can transform murine mammary epithelial cells as does wnt-1 cDNA, demonstrating a striking functional conservation between wg and wnt-1 (6). Recently, a receptor for Wg was identified to be a member of the Drosophila Frizzled (Fz, ref. 7) family of seven transmembrane receptors. DFz2, when introduced by transfection, confers recepient cells the ability to bind and to respond to Wg (8). The identification of Dfz2 as a Wg receptor implies that the fz family of genes, which include nine mammalian members identified so far (9, 10), are likely to encode receptors for Wnt molecules. However, the scarcity of soluble Wnt proteins (11) complicates the study of Wnt-Fz interactions and their specificity. We have developed an approach to address this issue using Xenopus embryos wothout soluble Wnt proteins.

In *Xenopus*, several Wnt molecules, such as Wg, Wnt-1, the Xenopus wnt-3A and wnt-8 (Xwnt-8), have been shown to mimic an early dorsalizing signal that induces the gastrula

organizer and in doing so are able to induce embryonic axis duplication (12-17), thus offering a unique opportunity to study Wnt function and Wnt signal transduction pathway. However, several other Wnt proteins, such as the Xenopus Wnt-5A, were found unable to induce axis duplication, and have been classified as a distinct subclass that activates a different pathway(s) (17-19).

In this report we show that Wnt-5A can induce axis duplication in the presence of a member of the mammalian Fz family, hFz5, but not other Fz proteins tested. Wnt-5A signaling via hFz5 is antagonized by GSK-3, and by the hFz5 N-terminal ectodomain that presumably binds and titrates Wnt-5A. These results identify hFz5 as a receptor for Wnt-5A. Importantly, because Wnt-5A is expressed during the development of the mammary gland (20), the revalation of Wnt-5A-hFz5 ligand-receptor relationship will enhance the understanding of normal mammary development and physiology. Moreover, this study establishes axis induction in *Xenopus* as a general approach to investigate Wnt-Fz relationships which play pivotal roles in mammmary neoplasia.

In addition, we also identified a novel secreted protein, FRP (for Frizzled related protein), as a functional antagonist to Wnt proteins. FRP shares significant homology with the amino-terminal region of Fz proteins but lacks any transmembrane domain. FRP can completely block axis duplication induced by Wg, Wnt-1 and Xwnt-8 in *Xenopus* embryos. Given the role of Wnt-1 as an oncogene in mammary cancer, the identification of FRP as an inhibitor of Wnt-1 may have implications on genetic intervention of tumor formation.

RESULTS AND DISCUSSION

hFz5 mediates Wnt-5A axis induction

Mouse Wnt-1, *Xenopus* wnt-3A (Xwnt-3A), Xwnt-8, and *Drosophila* Wg induce dorsal axis duplication when low levels, usually 1-10 pg, of their corresponding RNAs are injected into the ventral side of early *Xenopus* embryos (12-15, and data not shown). In contrast, Xwnt-5A RNA fails to do so even after ventral injection at higher doses (75pg to 1ng per embryo); instead, dorsal injection of Xwnt-5A RNA generates head and tail defects that may result from perturbation of cell movements during gastrulation (18). Xwnt-4 and Xwnt-11 behave similarly to Xwnt-5A (19). The Xwnt-8 dorsalizing function is observed before the mid-blastula transition (MBT) when zygotic transcription begins, whereas the Xwnt-5A effect occurs after MBT (13-15, 17). The difference between the effects of Xwnt-8 and Xwnt-5A may reflect the activation of distinct signaling pathways or the lack of a functional Xwnt-5A receptor(s) during axis formation.

To examine whether a particular Fz protein can function as an Xwnt-5A receptor, synthetic RNAs corresponding to Dfz2 (ref. 8) and six mammalian fz cDNAs - mfz3, 4, 6, 7, 8 (from mouse) and hfz5 (from human, ref. 10) - were pooled into two groups and co-injected with 10 pg of Xwnt-5A RNA into the ventral side of 4-cell stage embryos. Injection of Xwnt-5A alone, either fz group alone, or Xwnt-5A together with fz group 2 (mfz3, 4, 6, and 7) produced no phenotypic effects (Fig. 1A). However, co-injection of Xwnt-5A with fz group 1 RNAs (Dfz2, hfz5 and mfz8) induced extensive dorsal axis duplication; in many cases, duplication was complete as determined by the presence of

anterior structures such as the eyes and the cement gland (Fig.1A and 3A). When the three fz RNAs in group 1 were individually tested, Xwnt-5A plus hfz5 generated similar degrees of axis duplication, whereas Xwnt-5A plus Dfz2 or mfz8 did not (Fig. 1B). Thus, hFz5 alone among the Fz proteins tested is responsible for mediating axis induction by Xwnt-5A. The mature Wnt-5A proteins (after cleavage of the signal peptides) are 100% identical between mouse and human, and 95% identical between mouse and *Xenopus* (18, 21, 22). Given this high degree of sequence identity, it is not surprising that murine wnt-5A RNA also induced axis duplication when co-injected with hfz5 RNA, albeit less efficiently (Fig. 1C). The lower efficiency might be due to effects of untranslated regions in the murine wnt-5A construct on RNA stability and/or translation efficiency.

Axis duplication by Xwnt-5A plus fz group 1 or hfz5, as described above, was observed in fourteen of twenty embryo batches tested during the course of this study. In these cases, 90-100% of the injected embryos showed axis duplication, of which 17-82% included eyes. In the remaining six of twenty embryo batches, the same co-injection induced no or few axis duplications (less than 30%, none complete). The reason for this poor response in some batches is unknown. Possible explanations include variations in the stability of injected RNAs and/or translated proteins, the efficiency of Wnt-5A secretion, the assembly and/or localization of hFz5 protein, or the availability of unknown co-receptor molecules.

It should be noted that while hFz5 is more closely related to DFz2 and mFz8 than to any other known Fz proteins (8, 10), neither DFz2 nor mFz8 cooperated with Xwnt-5A in axis induction. However, DFz2 appeared to be functional in *Xenopus* embryos since the same concentration of Dfz2 RNA as used for co-injection with Xwnt-5A substantially enhanced axis induction by suboptimal amounts of wg RNA (data not shown). The failure

of the other Fz proteins to mediate Xwnt-5A function could be due to an inability to bind Xwnt-5A or an inability to signal in this context.

A dose response curve illustrated that in the presence of 0.4 ng hfz5 RNA per embryo, 1 pg Xwnt-5A RNA induced partial secondary axes whereas 20 pg of Xwnt-5A RNA sometimes hyperdorsalized embryos (Fig. 2). Although the relative protein levels have not been determined, the dose of Xwnt-5A RNA required for axis induction was in a similar range to effective doses of Xwnt-3A, Xwnt-8, wnt-1 and wg RNAs. Together these data suggest a high degree of specificity of the interaction between Xwnt-5A and hFz5.

Histological examination of embryos with duplicated axes revealed that Xwnt-5A plus hFz5 RNAs induced a full array of dorsal tissues, including notochord, neural tube and somites (Fig.3B). There is one notable difference between axes induced by Xwnt-8 and those induced by Xwnt-5A plus hFz5: while the ectopic axes induced by Xwnt-8 are often indistinguishable from the endogenous ones, the axes induced by Xwnt-5A and hFz5 are shorter in most cases, even when eyes and the cement gland are present (Fig. 3A). This might reflect the previously described ability of Xwnt-5A to inhibit cell movements during gastrulation (18).

Xwnt-8 and other Wnt proteins in its group induce dorsal development via the formation of an ectopic Spemann organizer (13-15). We tested whether Xwnt-5A plus hFz5 act similarly by examining the expression of the organizer-specific gene *goosecoid* (*gsc*, ref. 23), using wholemount in situ hybridization. Embryos injected with Xwnt-5A or hfz5 RNA alone expressed *gsc* only dorsally, as did uninjected controls; in contrast,

embryos co-injected with Xwnt-5A and hfz5 RNAs exhibited two opposing domains of *gsc* expression, indicating the formation of an ectopic organizer (Figure 3C).

Drosophila Wg function is mediated by inhibition of the *zeste-white 3 (shaggy)* gene product, the homolog of vertebrate glycogen synthase kinase-3 (GSK-3, ref. 24). Likewise, the dorsalizing function of Xwnt-8 RNA in *Xenopus* is mimicked by dominant-negative mutant forms of GSK-3 and antagonized by overexpression of wild type GSK-3 (25-27). We therefore asked whether Xwnt-5A signaling via hFz5 is also transduced by inhibition of GSK-3. Co-injection of wild type GSK-3β RNA blocked dorsal axis duplication by Xwnt-5A plus hfz5 RNAs (Fig. 4A), suggesting that Xwnt-5A signaling through hFz5 requires the inhibition of GSK-3.

All Fz proteins contain a N-terminal extracellular domain composed of a conserved cysteine-rich domain (CRD) and a variable linker region before the first putative transmembrane helix (10). The N-terminal ectodomain of DFz2, when anchored to the membrane, promoted binding of Wg to the cell surface, and deletion of CRD from mFz4 abolished Wg binding (8). Thus the N-terminal extracellular domain appears involved in ligand binding. If the N-terminal ectodomain of hFz5 is involved in binding Xwnt-5A, its overexpression as a secreted molecule might prevent Xwnt-5A from binding to and signaling through hFz5. Indeed, co-injection of RNA coding for the hFz5 N-terminus (hFz5N), without provision of a membrane anchor, antagonized axis induction by Xwnt-5A plus hFz5 in a dose-dependent manner (Fig. 5). Dorsal injection of hfz5N RNA alone did not affect endogenous dorsal axis formation (although gastrulation defects were seen in some embryos); the injected embryos appeared to have an intact neural groove at the neurula stage, and the anteriormost structures (eyes and the cement gland) were present at

the tadpole stage (not shown). These results argue for a specific effect of hFz5N on blocking axis duplication by Xwnt-5A plus hFz5 .

FRP: an antagonist of Wnt signaling

While we were studying Wnt-5A-hFz5 interactions, Dr. Jeff Rubin at NCI purified a secreted polypeptide from conditioned media of a human cell line. cDNA cloning revealed that this peptide shares significant homology with the cysteine-rich domain (CRD) of the amino-terminal region of Fz proteins but lacks any transmembrane domains (Fig.6). This protein, therefore, was named Frizzled related protein (FRP). We and Dr. Rubin initiated a collaboration to study the function of this novel secreted protein.

From the study on Wg-DFz2 (8) and our study on Wnt-5A-hFz5 as described above, it appears that the amino-terminal region of Fz proteins are involved in binding to Wnt ligands. Since FRP is homologous to this region, we reasoned that it may bind to and modulate the signaling activity of Wnt proteins. We envisioned two alternatives: FRP might antagonize Wnt function by binding the protein and blocking access to its cell surface receptor such as Fz proteins; or FRP might enhance Wnt activity by facilitating the presentation of ligand to the Fz receptors, analogous to the action of soluble receptors for certain cytokines (28).

To test these possibilities, we examined the effect of FRP on Wnt-dependent axis duplication. As shown in Figure 7, injection of Wnt-1, Wg or Xwnt-8 mRNA induced partial or complete axis duplication in at least 75% of the embryos. However, when similar quantities of FRP and Wnt RNA were co-injected, the incidence and extent of axis duplication were significantly reduced. Higher concentration of FRP RNA resulted in

almost complete inhibition of Wnt signaling (Fig. 7). It should be noted that FRP inhibited axis duplication by sub-optimal amount of Wnt-1 which induces mostly partial axis duplication. This suggests that even under the sub-optimal condition of Wnt signaling, FRP does not enhance the signaling thus does not function as a soluble "co-receptor" but rather behaves as an antagonist. Surprisingly, FRP was much less effective in antagonizing Xwnt-3A (Fig. 7), suggesting a degree of specificity regarding interactions of FRP with different members of the Wnt family.

CONCLUSIONS

Our data indicate that in the presence of hFz5, Wnt-5A can transduce a signal similar to that of Xwnt-8 or Wnt-1, mediated by a pathway involving down-regulation of GSK-3. The simplest interpretation of these results is that hFz5 functions as a receptor for Wnt-5A.

We further demonstrated that FRP, a molecule related to the Fz extracellular domain, functions as an antagonist for Wnt signal transduction. Given that certain wnt genes, such as wnt-1, are oncogenic, this raises the possibility that FRP may function as a anti-oncogene or tumor suppresser gene.

At least sixteen *wnt* and eight *fz* , and four FRP genes (29) have been identified in mammals thus far. The ligand-receptor relationships among Wnt and Fz and FRP proteins are likely to be complex, as seen in other large families of signaling molecules and their receptors. Biochemical study of the Wnt-Fz interaction remains challenging because of the difficulty in obtaining soluble Wnt proteins. By reconstituting a Wnt-Fz signaling system in *Xenopus* embryos and blocking this signaling system via a dominant-negative form of Fz ectodomain or FRP, the experiments described here provide a general assay to address the relationships among Wnt, Fz and FRP proteins.

FIGURE LEGENDS

Figure 1. Dorsal axis induction by Xwnt-5A mediated via hFz5. n represents the total number of embryos scored from two to six independent experiments; each bar represents the average percentage of axis duplication; the solid portion within each bar represents the average percentage of extensive axis duplication, which is defined by the presence of the cement gland and at least one eye in the duplicated axis. Unless otherwise specified, 10 pg of wnt RNA and/or 400 pg of each fz RNA were injected per embryo. (**A**) Ventral injection of Xwnt-5A RNA together with RNA for fz group 1 (Dfz2, hfz5 and mfz8) induces axis duplication. Xwnt-5A RNA alone, fz group 1 or group 2 (mfz3, 4, 6, and 7) alone, and Xwnt-5A RNA plus fz group 2 did not. (**B**) Xwnt-5A RNA induces axis duplication in the presence of hfz5 RNA, but not with Dfz2 or mfz8 RNA. (**C**) Murine wnt-5A RNA plus hFz5 also induce axis duplication.

Figure 2. Dose response curve of Xwnt-5A and hfz5 RNAs for axis duplication. The bar legend is as in Figure 1.

Figure 3. Induction of anteriormost structures, dorsal axial tissues, and the Spemann organizer by Xwnt-5A plus hfz5. (**A**) Xwnt-5A plus hfz5 induce axis duplication. An example of complete axis duplication with eyes in both axes is shown (stage 41). (**B**) Xwnt-5A plus hfz5 induce a complete set of dorsal tissues. Cross section in the trunk region of an embryo at stage 41 reveals the presence of a neural tube, notochord and

somites in both axes. (**C**) Xwnt-5A plus hfz5 induce ectopic *goosecoid (gsc)* expression in embryos at stage 10.5, as visualized by whole mount *in situ* hybridization.

Figure 4. GSK-3β antagonizes axis induction by Xwnt-5A plus hfz5. RNA for human GSK-3β was injected at 1ng per embryo. (**A**). Examples of control (uninjected) and injected embryos at stage 19. (**B**) Note the complete suppression of axis duplication in the presence of GSK-3. The Bar legend is as in Fig. 1.

Figure 5. The N-terminal ectodomain of hFz5 (hFz5N) as a secreted protein suppresses axis duplication by Xwnt-5A plus hfz5 in a dose dependent manner. Dorsal injection of same doses of hfz5N RNA did not affect the endogenous dorsal development (data not shown). At higher doses (0.8-2ng RNA per embryo) some embryos with gastrulation defects were observed.

Figure 6. Comparison of the cysteine-rich domain (CRD) of FRP and other members of the Fz family. Solid black shading highlights identities present in FRP and any other Fz family member. The consensus sequence indicates residues present in at least 8 of the 16 Fz or Fz-related proteins. **, the ten invariant cysteine residues; *, other invariant residues.

Figure 7. The hypothetical structures of Fz and FRP proteins. CRD: cysteine-rich domain.

Figure 8. FRP inhibits axis duplication induced by Wnt-1, Wg or Xwnt-8. Note that FRP is much less effective in inhibiting Xwnt-3A. The Bar legend is as in Fig. 1. The amount of mRNA injected per embryo is shown below the bars.

METHODS

Plasmids

All fz cDNAs were subcloned in the pRK5 vector with a optimal Kozak consensus sequence for translation at the initiator ATG (CCACCATG, preceded by different restriction sites for subcloning), and with different lengths of 3' untranslated regions. Xwnt-5A and human GSK-3 were in pSP64 vector as described (18, 26).Murine wnt-5 cDNA (ref. 21) was cloned in pCS2+ vector as the EcoRI and XbaI fragment, which contains about 60 bp 5' and 360 bp 3' untranslated regions. hfz5N was generated by introduction of a stop codon just before the first putative transmembrane helix (changing the preceding amino acids 237 and 238 from phenylalanine and trytophan to threonine and arginine). The corresponding DNA fragment was subcloned in pCS2+. The FRP NaeI-SalI cDNA fragment, which includes the full coding sequence, was subcloned into the StuI and XhoI sites of pCS2+.

RNA injection

All RNAs for injection were synthesized as capped transcripts *in vitro* with SP6 RNA polymerase (Ambion Megascript). Unless otherwise specified, RNAs were injected into the two cells near the equatorial midline region at the 4-cell stage.

Embryo handling

Embryo preparation, staging, fixation and sectioning, and wholemount in situ hybridization were performed as described (26).

Scoring axis duplication

Percentages of axis duplication were obtained by dividing the total number of embryos with a duplicated axis by the total number of embryos, scored from two to six independent experiments. Extensive axis duplication is defined by the presence of the cement gland and at least one eye in the duplicated axis.

REFERENCE

1. Nusse R, Varmus HE: Wnt genes. Cell 69: 1073-87 (1992).

2. Parr BA, McMahon AP: Wnt genes and vertebrate development. Curr Opin Genet Dev 4(4):523-8 (1994).

3. Tsukamoto AS, Grosschedl R, Guzman RC, Parslow T, Varmus HE: Expression of the int-1 gene in transgenic mice is associated with mammary gland hyperplasia and adenocarcinomas in male and female mice. Cell 55: 619-25 (1988).

4. Klingensmith J, Nusse R: Signaling by wingless in Drosophila. Dev Biol 166: 396-414 (1994).

5. Siegfried E, Perrimon N: Drosophila wingless: a paradigm for the function and mechanism of Wnt signaling. Bioessays 16: 395-404 (1994).

6. Ramakrishna NR, Brown AMC: Wingless, the Drosophila homolog of the proto-oncogene Wnt-1, can transform mouse mammary epithelial cells. Development (suppl): 95-103 (1993).

7. Vinson CR, Conover S, Adler PN: A Drosophila tissue polarity locus encodes a protein containing seven potential transmembrane domains. Nature 338: 263-4 (1989).

8. Bhanot P, Brink M, Samos CH, Hsieh J-C, Wang Y, Macke JP, Andrew D, Nathans J, Nusse R: A new member of the frizzled family from Drosophila functions as a Wingless receptor. Nature 382: 225-230 (1996).

9. Chan SDH, Karpf DB, Fowlkes ME, Hooks M, Bradley MS, Voung V, Bambino T, Liu, MY, Arnaud CD, Strewler GJ, Nissenson RA: Two homologs of the Drosophila polarity gene frizzled (fz) are widely expressed in mammalian tissues. J Biol Chem 267: 25202-207 (1992).

10. Wang Y, Macke JP, Abella BS, Andreasson K, Worley P, Gilbert DJ, Copeland NG, Jenkins NA, Nathans J: A large family of putative transmembrane receptors homologous to the product of the Drosophila tissue polarity gene frizzled. J Biol Chem 271: 4468-76 (1996).

11. Smolich BD, McMahon JA, McMahon AP, Papkoff J: Wnt family proteins are secreted and associated with the cell surface. Mol Biol Cell 4: 1267-75 (1993).

12. McMahon A, Moon RT: Ectopic expression of the proto-oncogene int-1 in Xenopus embryos leads to duplication of the embryonic axis. Cell 58: 1075-84 (1989).

13. Smith WC, Harland RM: Injected Xwnt-8 RNA acts early in Xenopus embryos to promote formation of a vegetal dorsalizing center. Cell 67: 753-65 (1991).

14. Sokol S, Christian JL, Moon RT, Melton DA: Injected wnt RNA induces a complete body axis in Xenopus embryos. Cell 67: 741-52 (1991).

15. Chakrabarti A, Matthews G, Colman A, Dale L: Secretory and inductive properties of Drosophila wingless protein in Xenopus oocytes and embryos. Development 115: 355-369 (1992).

16. Wolda SL, Moody CJ, Moon RT: Overlapping expression of Xwnt-3A and Xwnt-1 in neural tissue of Xenopus laevis embryos. Devel Biol 155: 46-57 (1993).

17. Moon RT, Christian JL, Campbell RM, McGrew LL, DeMarais AA, Torres M, Lai CJ, Olson DJ, Kelly GM: Dissecting Wnt signalling pathways and Wnt-sensitive developmental processes through transient misexpression analyses in embryos of Xenopus laevis. Development (Suppl): 85-94 (1993)

18. Moon RT, Campbell RM, Christian JL, McGrew LL, Shih J, Fraser S: Xwnt-5A: a maternal Wnt that affects morphogenetic movements after overexpression in embryos of Xenopus laevis. Development 119: 97-111 (1993).

19. Du SJ, Purcell SM, Christian JL, McGrew LL, Moon RT: Identification of distinct classes and functional domains of Wnts through expression of wild-type and chimeric proteins in Xenopus embryos. Mol Cell Biol 15: 2625-34 (1995).

20. Gavin B, McMahon AP: Differential regulation of the Wnt gene family during pregnancy and lactation suggests a role in postnatal development of the mammary gland. Mol Cell Biol 12: 2418-23 (1992).

21. Gavin B, McMahon JA, McMahon AP: Expression of multiple novel Wnt-1/int-1 related genes during fetal and adult mouse development. Genes Dev 4: 2319-32 (1990).

22. Clark CC, Cohen I, Eichstetter I, Cannizzaro LA, McPherson JD, Wasmuth JJ, Iozzo RV: Molecular cloning of the human proto-oncogene Wnt-5A and mapping of the gene (WNT5A) to chromosome 3p14-p21. Genomics 18: 249-60 (1993)

23. Cho KWY, Blumberg B, Steinbeisser H, DeRobertis, EM: Molecular nature of Spemann's organizer: the role of the homeobox gene goosecoid. Cell 67: 1111-1120 (1991).

24. Siegfried E, Chou TB, Perrimon N: wingless signaling acts through zeste-white 3, the Drosophila homolog of glycogen synthase kinase-3, to regulate engrailed and establish cell fate. Cell 71: 1167-79 (1992).

25. Pierce SB, Kimelman D: Regulation of Spemann organizer formation by the intracellular kinase Xgsk-3. Development 121: 755-65 (1995).

26. He X, Saint-Jeannet JP, Woodgett JR, Varmus HE, Dawid IB: Glycogen synthase kinase-3 and dorsoventral patterning in Xenopus embryos. Nature 374: 617-622 (1995).

27. Dominguez I, Itoh K, Sokol SY: Role of glycogen synthase kinase 3 beta as a negative regulator of dorsoventral axis formation in Xenopus embryos. Proc Natl Acad Sci USA 92: 8498-502 (1995).

28. Kishimoto T, Tada H, Akira S: Cytokine signaling. Cell 76: 253-62 (1994).

29. Rattner A, Hsieh J-C, Smallwood PM, Gilbert DJ, Copeland NG, Jenkins NA, Nathans J: A family of secreted proteins contains homology to the cysteine-rich ligand-binding domain of frizzled receptors. Proc Natl Acad Sci USA 94: 2859-63 (1997).

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BIBLIOGRAPHY

Publications

He, X., Saint-Jeannet, J-P., Wang, Y., Nathans, J., Dawid, I.B., and Varmus, H. E. 1997. A member of the Frizzled protein family mediating axis induction by Wnt-5A. Science 275: 1652-4.

Finch, P.W., **He, X.**, Kelley, M.J., Uren, A., Schaudies, P., Popescu, N.C., Rudikoff, S., Aaronson, S.A., Varmus, H.E., and Rubin, J. S. (1997). Purification and molecular cloning of a secreted, Frizzled-related antagonist of Wnt action. Proc. Natl. Acad. Sci. USA 94: 6770-5.

Saint-Jeannet, J-P., **He, X.**, Varmus, H. E., and Dawid, I. B. 1997. Dorsoventral patterning of the neuraxis: induction of neural crest by Wnt-1 and Wnt-3A. Proc. Natl. Acad. Sci. USA, in press.

Meeting abstract

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Figure 1 (He et al.)

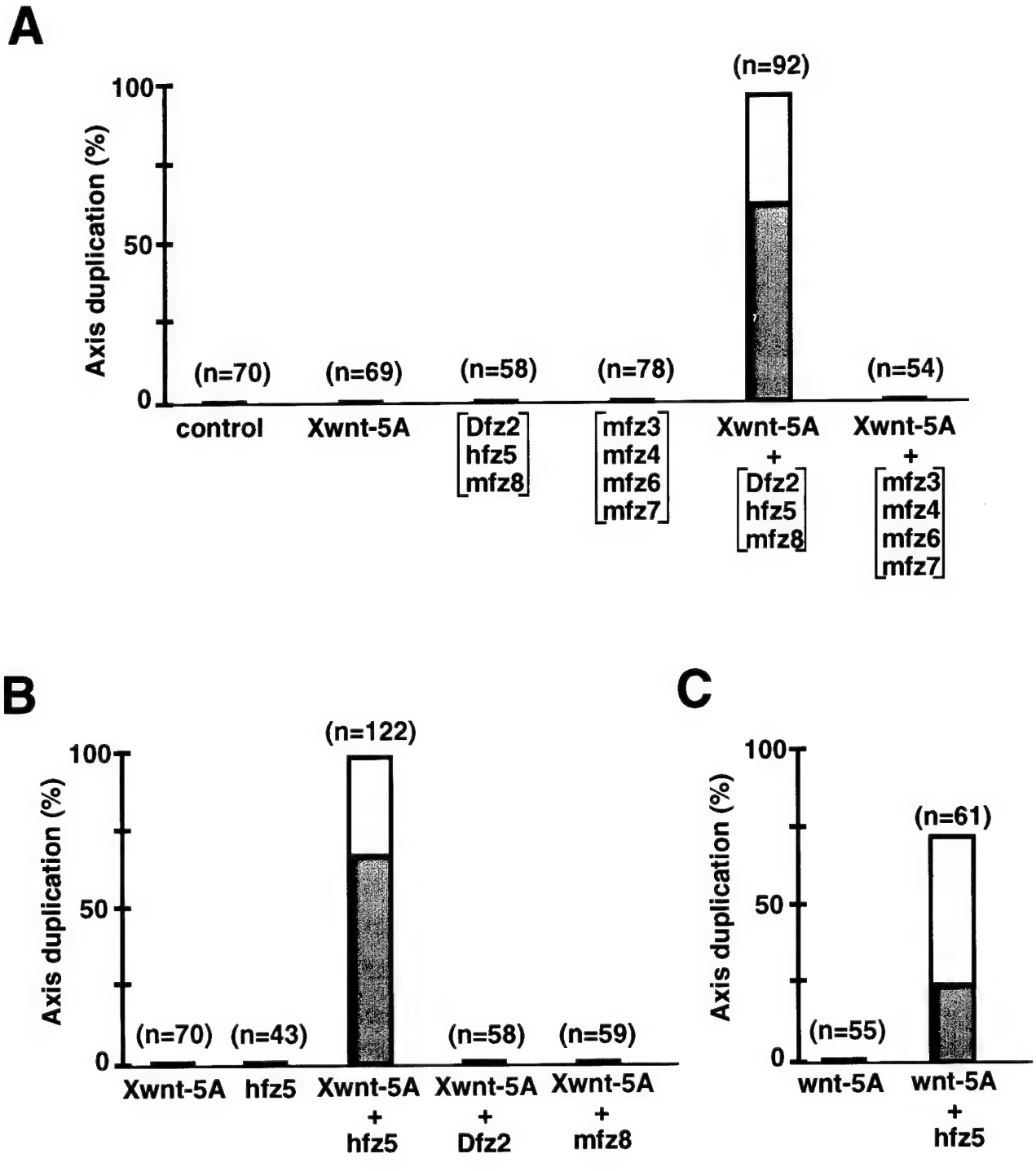


Figure 2 (He et al.)

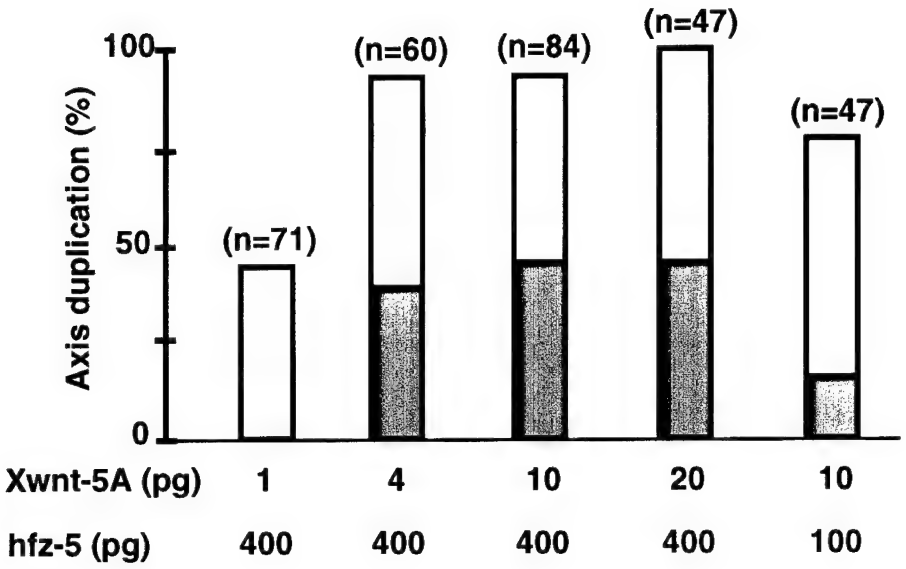


Figure 3 (He et al.)

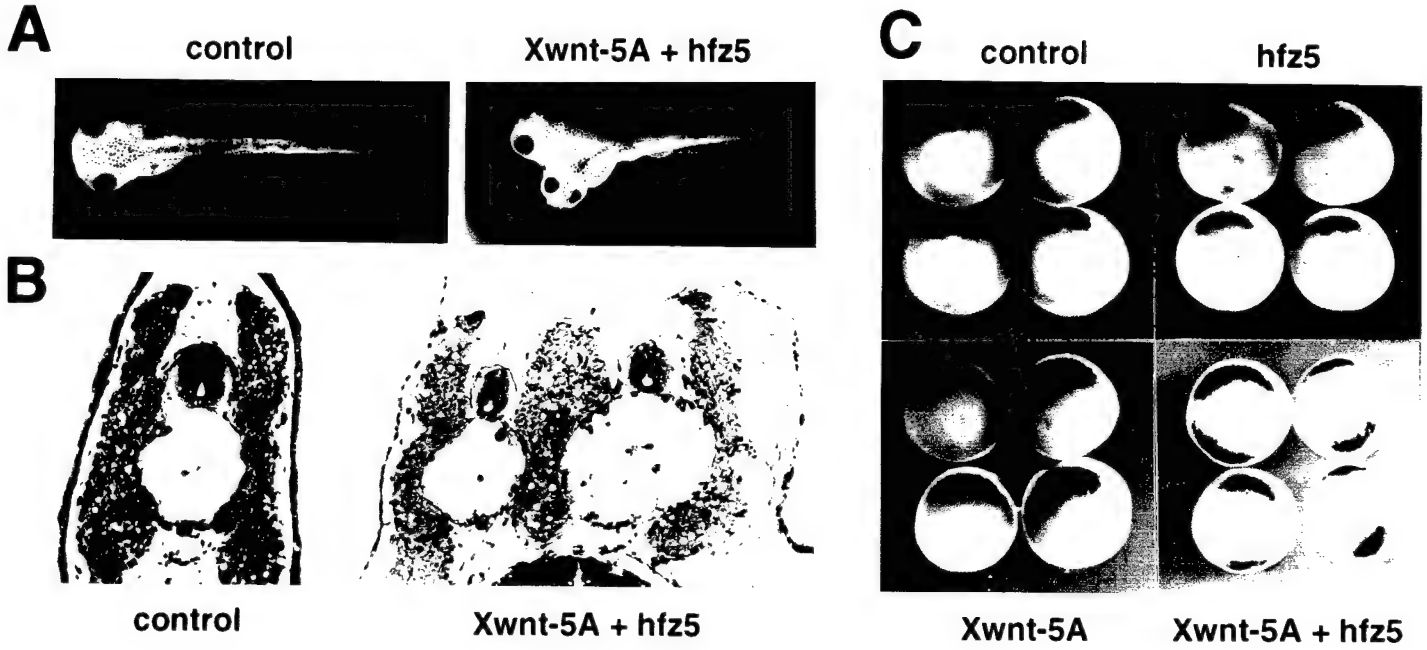


Figure 4 (He et al.)

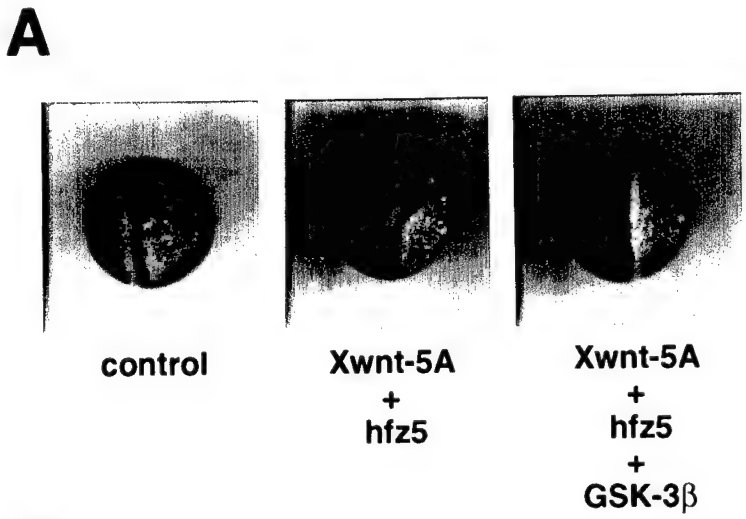
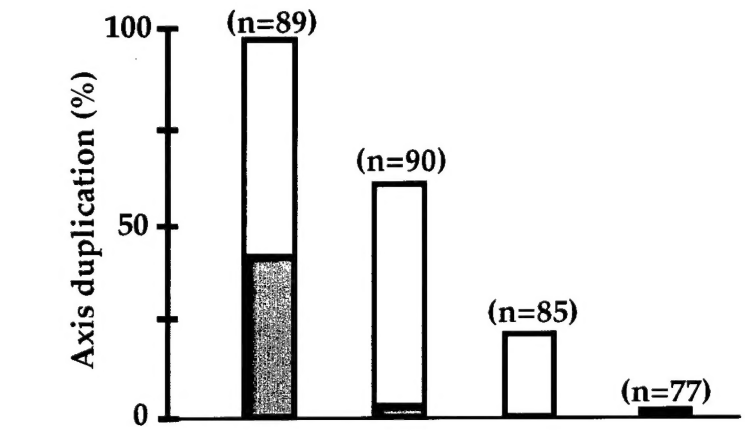
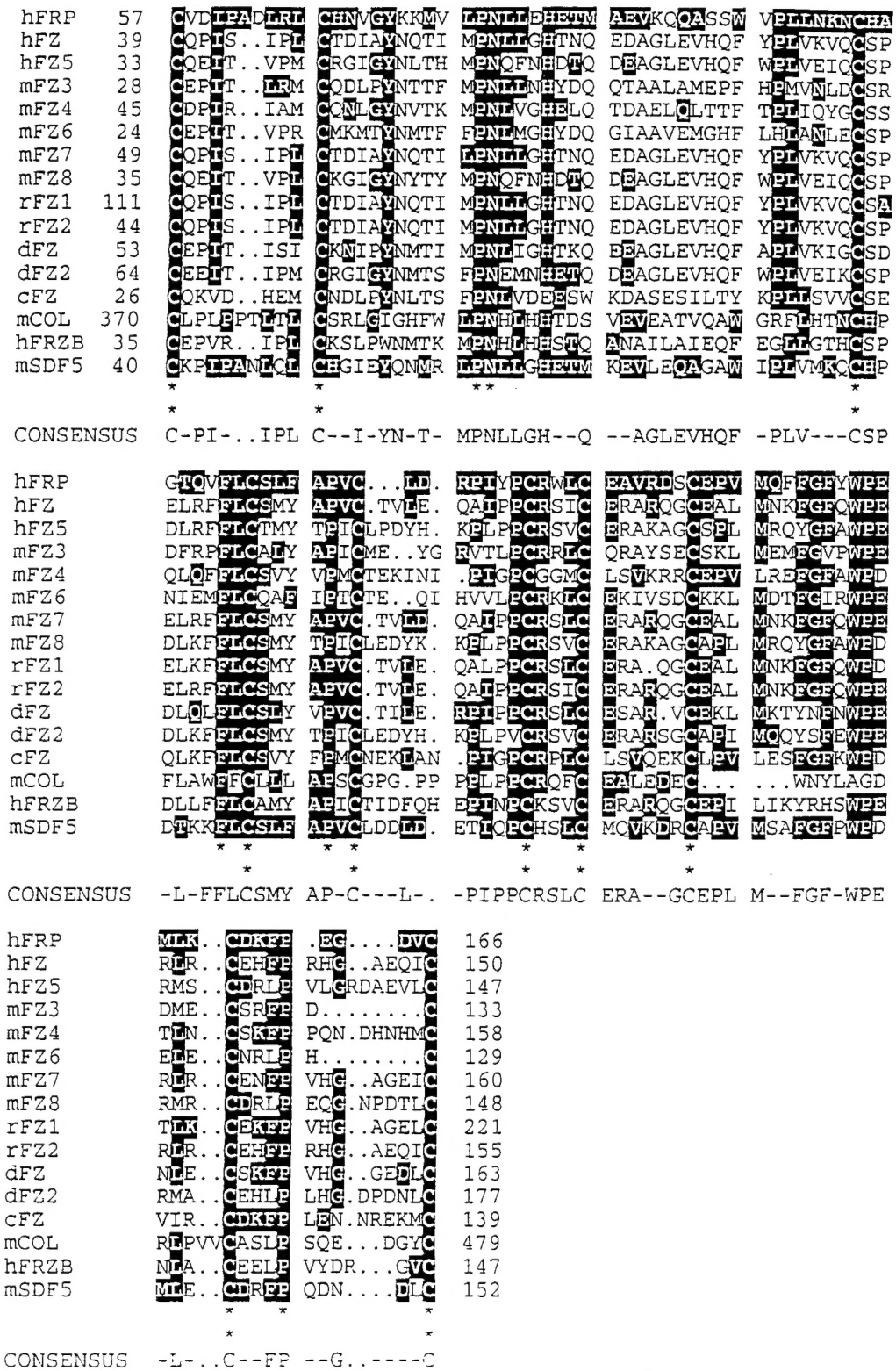


Figure 5 (He et al.)



Xwnt-5A (pg)	10	10	10	10
hfz-5 (pg)	400	400	400	400
hfz-5N (pg)	0	400	800	2000

Figure 6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rFZ1 111 CQPIS..IPL CTDIAYNQTI MPNLLGHTNQ EDAGLEVHQF YPLVKVQCSA
rFZ2 44 CQPIS..IPL CTDIAYNQTI MPNLLGHTNQ EDAGLEVHQF YPLVKVQCSP
dFZ 53 CEPIT..ISI CKNIPYNMTI MPNLIGHTKQ EEAGLEVHQF APLVKIGCSD
dFZ2 64 CEPIT..IPM CRGIGYNMTS FPNEMNHETQ DEAGLEVHQF WPLVEIKCSP
cFZ 26 CQKVD..HEM CNDLPYNLTS FPNLVDEESW KDASESILTY KPLLSVVCSE
mCOL 370 CLPLPPTLTL CSRLGIGHFW LPNHLHHTDS VEVEATVQAW GRFLHTNCHP
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mSDF5 40 CKPIPANLQL CHGIEYQNMR LPNLLGHETM KEVLEQAGAW IPLVMKQCHP
CONSENSUS C-PI-..IPL C--I-YN-T- MPNLLGH--Q --AGLEVHQF -PLV---CSP
hFRP GTQVFLCSLF APVC...LD. RPIYPCRWLC EAVRDSCEPV MQFFGFYWPE
hFZ ELRFFLCSMY APVC.TVLE. QAIPPCRSIC ERARQGCEAL MNKFGFQWPE
hFZ5 DLRFFLCTMY TPICLPDYH. KPLPPCRSVC ERAKAGCSPL MRQYGFAWPE
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hFRZB DLLFFLCAMY APICTIDFQH PPINPCKSVC ERARQGCEPI LIKYRHSWPE
mSDF5 DTKKFLCSLF APVCLDDLD. ETIQPCHSLC MQVKDRCAPV MSAFGFPWPD
CONSENSUS -L-FFLCSMY AP-C---L-. -PIPPCRSLC ERA--GCEPL M--FGF-WPE
hFRP MLK..CDKFP .EG....DVC 166
hFZ RLR..CEHFP RHG..AEQIC 150
hFZ5 RMS..CDRLP VLGRDAEVLC 147
mFZ3 DME..CSRFP D........C 133
mFZ4 TLN..CSKFP PQN.DHNHMC 158
mFZ6 ELE..CNRLP H........C 129
mFZ7 RLR..CENFP VHG..AGEIC 160
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rFZ1 TLK..CEKFP VHG..AGELC 221
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dFZ NLE..CSKFP VHG..GEDLC 163
dFZ2 RMA..CEHLP LHG.DPDNLC 177
cFZ VIR..CDKFP LEN.NREKMC 139
mCOL RLPVVCASLP SQE...DGYC 479
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CONSENSUS -L-..C--FP --G..----C

Figure 7

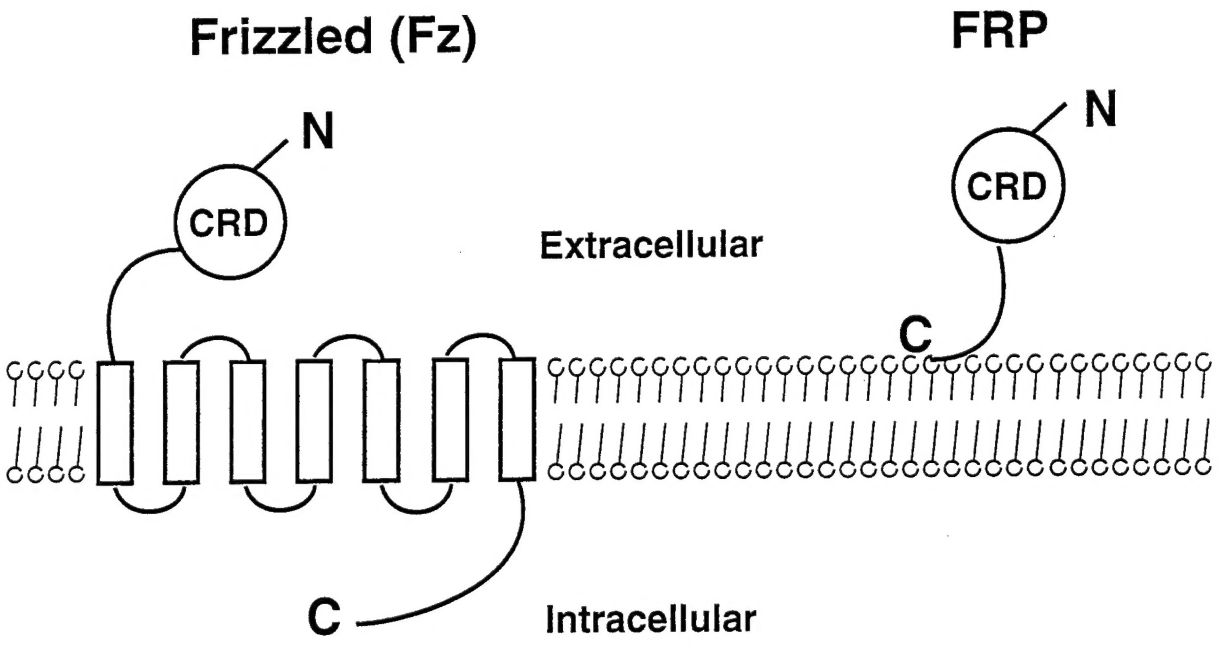
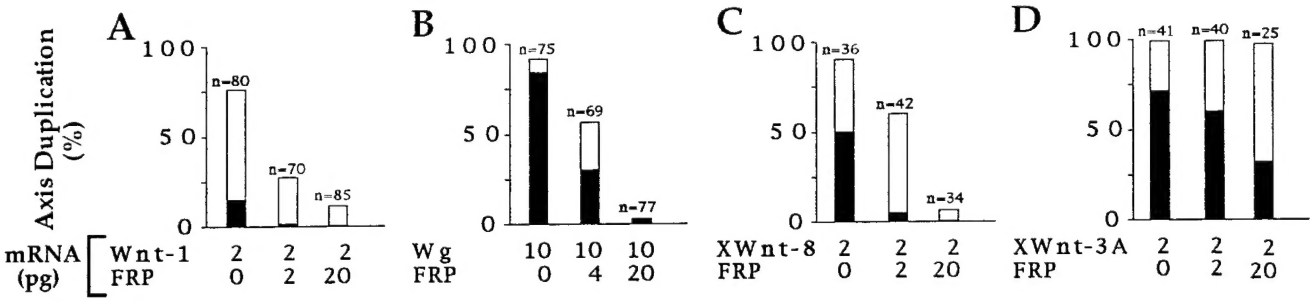


Figure 8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A
100
50
0
n=80
n=70
n=85
mRNA (pg)
Wnt-1 2 2 2
FRP 0 2 20
B
100
50
0
n=75
n=69
n=77
Wg 10 10 10
FRP 0 4 20
C
100
50
0
n=36
n=42
n=34
XWnt-8 2 2 2
FRP 0 2 20
D
100
50
0
n=41
n=40
n=25
XWnt-3A 2 2 2
FRP 0 2 20